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# Population-level effects in *Amphiascus tenuiremis*: Contrasting matrix- and individual-based population models

Elin Lundström Belleza [a], Markus Brinkmann [b], Thomas G. Preuss [c], Magnus Breitholtz [a,*]

[a] *Stockholm University, Department of Applied Environmental Science (ITM), Svante Arrhenius väg 8, S-106 91 Stockholm, Sweden*
[b] *RWTH Aachen University, ABBt – Aachen Biology and Biotechnology, Institute for Environmental Research, Department of Ecosystem Analysis, Worringerweg 1, D-520 74 Aachen, Germany*
[c] *RWTH Aachen University, ABBt – Aachen Biology and Biotechnology, Institute for Environmental Research, Chair of Environmental Biology and Chemodynamics, Worringerweg 1, D-520 74 Aachen, Germany*

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ABSTRACT

Environmental risk assessment (ERA) is generally based on individual-level endpoints, even though protection goals in ERA intend higher biological levels. Population models have the potential to translate individual-level endpoints to population-level responses and range from simple demographic equations to highly complex individual based models (IBMs). The aims of the current study were to develop a matrix model (MM) with the structure and parameterization proposed in the draft OECD guideline "Harpacticoid copepod development and reproduction test with *Amphiascus tenuiremis*", and an IBM with the same data requirements. Experimental data from lindane exposure from validation studies of the OECD guideline was projected to the population level. Lindane does not only cause effects on survival and reproduction, but also on the time it takes to develop from larvae to adults. The two model approaches were contrasted in terms of their ability to properly project these effects on development. The MM projected smaller effects of the lindane treatments on population growth rate compared to the IBM since in its proposed structure, it did not include the delay in development explicitly. Population-level $EC_{10}$ for population growth rate in the IBM was at the same level as the most sensitive individual-level endpoint, whereas the $EC_{10}$ from the MM was not as sensitive. Based on these findings, our conclusion is that the IBM (or an improved MM) should be used for datasets including shifts in development, whereas the simpler MM is sufficient for datasets where only mortality and reproduction are affected, or as a screening tool in lower-tier population-level ERA.



## 1. Introduction

Protection goals of environmental (ecological) risk assessment (ERA) are generally focused on populations, communities and ecosystems (European Commission, 2002; van Leeuwen and Vermeire, 2007). However, risk assessors commonly use relatively basic standard laboratory test data focusing on individual-level apical endpoints, e.g. effects on growth, reproduction or survival (European Commission, 2003). As a consequence, effects on higher organizational levels, i.e. the level of protection, are often neglected (Forbes et al., 2008; Galic et al., 2010). Therefore, there is a need for more population-level oriented approaches within the field of ERA (e.g. Barnthouse et al., 2008; Forbes et al., 2010).

The traditional way of assessing environmental risks is often by selecting the most sensitive individual-level endpoints from standard ecotoxicological tests (e.g. European Commission, 2003). The obtained value is then divided by uncertainty factors to reflect, for example (i) intra- and inter-species variations, (ii) to account for the extrapolations from short-term responses to long-term consequences and (iii) from laboratory results to the field (OECD, 2011a). As an alternative, Forbes et al. (2001) have suggested that endpoints used in ERA should integrate age- or stage-specific survival, reproductive endpoints, and development, to describe changes in population density through time, e.g. population growth rate. A possible solution is population models that have the potential for adding relevance to ERA by extrapolating individual-level effects to the population-level, and by incorporating ecological complexity in a way that informs the environmental management process (Forbes et al., 2010). The use of population models can hereby translate individual-level test endpoints into the higher-level protection goals of ERA (Hommen et al., 2010). Population models are

* Corresponding author. Tel.: +46 8 674 7241; fax: +46 8 674 7325.
*E-mail address:* magnus.breitholtz@itm.su.se (M. Breitholtz).

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mentioned with increasing frequency in European directives and guidance documents on ERA (e.g. EFSA, 2009, 2010; SCENIHR, 2012; EFSA, 2013, 2014), in addition, much work has been done within the scientific community (e.g. Barnthouse et al., 2008; Galic et al., 2010; Pastorok et al., 2002). Individual-level test data have previously been used in matrix models (MMs) and individual based models (IBMs) to assess the toxicity of different substances (e.g. MMs: Klok and de Roos, 1996; Lin et al., 2005; Raimondo and McKenney, 2006, IBMs: Baveco and De Roos, 1996; Preuss et al., 2010; Gabsi et al., 2014). Both approaches make it possible to extrapolate from the individual-level (where effects are measured) to the population-level (the level of protection) (Grimm et al., 2009). Population growth rate is commonly thought of as the key variable that links individual-level effects to the population-level (e.g. Calow et al., 1997; Caswell, 2001). The intrinsic or instantaneous rate of increase ($r$) expresses population growth, and is called the population growth rate. Population density is stable when $r = 0$, and declining populations are defined by $r$ values <0 (e.g. Sibly, 1999).

MMs group individuals into a number of classes based on age, size or development stage. The parameters of the model (survival rates, or life stage transitions, and fecundities) are calculated from an experimental population, e.g. under influence of test chemicals, and form a matrix (Caswell, 2001), from which population growth rate is derived. A stage-based Lefkovitch matrix model is recommended for projection of the test results in the draft guideline "Harpacticoid copepod development and reproduction test with *Amphiascus tenuiremis*" (OECD, 2013). It has been demonstrated that MMs with the proposed structure are capable of projecting effects on survival and reproduction well but are not able to capture effects on development, i.e. the time it takes the animals to develop to the next stage (Chandler et al., 2004). Although the structure and parameterization of the MM could be modified to include these effects, an alternative would be the use of IBMs, which include each individual in a population and describe the individual responses. As in real populations in the environment, population-level responses in IBMs emerge from the response of the individuals (DeAngelis and Grimm, 2014).

Due to the different levels of complexity and potentially also data requirements of the different modeling approaches, population models are currently not frequently put into practice in ERA, and stakeholders name, e.g. the lack of guidance on how to choose and use them as reason for this (Hunka et al., 2013). To overcome this uncertainty, Meli et al. (2014) proposed that contrasting population models of differing complexity may aid risk assessors in choosing what population model to use. The usefulness of this approach has been demonstrated by a number of further studies. Topping et al. (2005) compared a simple life-history model and an individual based landscape model (IBLM) for Skylark populations exposed to a pesticide. Individual-level effects for the springtail *Folsomia candida* exposed to copper sulfate were projected to the population level using a MM and a more complex IBM (Meli et al., 2014). A study focusing on population level risk to fish from different toxicants used MMs of different complexities and compared them to risk-estimates based on individual-level effects (Hanson and Stark, 2012). Generally, more complex models were found to be more sensitive for detecting population-level responses, but models of differing complexities had different advantages in terms of e.g. input-data requirements and in communicating the results from the models.

The aims of the current study were: (i) to develop a MM with the structure and parameterization proposed in the draft OECD guideline and an IBM with the same data requirements for the harpacticoid copepod *A. tenuiremis*, (ii) to project experimental data that was part of the validation studies for the OECD guideline "Harpacticoid copepod development and reproduction test" to the population level and (iii) to contrast the two model approaches in terms of their ability to properly project effects on development.

## 2. Materials and methods

### 2.1. Test species

The harpacticoid copepod *A. tenuiremis* molts and sheds an exoskeleton between each developmental stage. It has six naupliar stages (NI–NVI) and six copepodite stages (CI–CV + A; the reproducing adult stage). General information on the morphology and ecology of *A. tenuiremis* has been described in e.g. Lang (1948). *A. tenuiremis* as a test species has been described in e.g. Coull and Chandler (1992) and Chandler et al. (2004).

### 2.2. Population models

The stage-based Lefkovitch MM used in the current study was previously used in Lundström et al. (2010) and is based on the recommendations in the OECD draft guideline "Harpacticoid copepod development and reproduction test" (OECD, 2013). In a previous study, an IBM was developed for the harpacticoid copepod *Nitocra spinipes* (Preuss et al., 2011). Since *A. tenuiremis* and *N. spinipes* are two closely related species that share life cycles, the IBM developed for *N. spinipes* was, after recalibration of parameter values, used also for *A. tenuiremis* in the present study. The different model approaches were used to assess data on the effect of lindane that was part of the validation studies for the OECD draft guideline on population growth rate, and projected population dynamics over two "generations". Here, one generation was defined as the time it took newly hatched nauplii to develop into females and having two viable clutches of offspring. In the draft guideline, the test duration is stated as up to 36 days (OECD, 2013).

MMs are mathematical structures that are filled with life table information from e.g. a life-cycle test, Fig. 1A. Both control populations and exposed populations are modeled. Input data used in the *A. tenuiremis* MM are found in Table A.1. IBMs are built based on the life cycle of the species and parameterized on control experiments, so that the normal life cycle can be implemented (Fig. 1B). Life-history variables from animals exposed to a toxicant can then be simulated in the IBM. Parameters and variables for the *A. tenuiremis* IBM can be found in Tables A.2 and A.3.

### 2.3. Datasets

The *A. tenuiremis* IBM was based on the data for controls obtained in two independent life cycle experiments (Chandler et al., 2004; OECD, 2011b). Raw data from a life cycle test with *A. tenuiremis* exposed to the pesticide lindane (model substance) at measured concentrations of 2.2 (±0.8), 4.7 (±2.0), 7.6 (±3.4), 11.6 (±3.8), 15.7 (±7.3) $\mu g\,L^{-1}$ was used in both model approaches. The raw data originated from Stockholm University's part of a validation test for the OECD test guideline "Harpacticoid copepod development and reproduction test" (OECD, 2011b).

In short, newly hatched nauplii aged <24 h, were placed individually in separate wells on 96-well microplates containing control- and test-media. On each microplate, 20 wells were used, three microplates per treatment, resulting in 60 starting animals. Nauplii were allowed to develop into copepodites and subsequently adults. The days on which the animals reached the first copepodite stage and the sexually reproducing copepodite (adult) stage were recorded. Random male:female mating pairs were allocated in 24-well microplates. The number of nauplii from two consecutive clutches was recorded. The experiment was terminated when >80% of the control pairs had their second clutch. The test media was formulated from 30‰ synthetic seawater, and was renewed every